Habitat Selection of Laboratory Populations of Tribolium [1]

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Part I: Review of the Ecology of Flour Beetles

The effect of ecological factors, such as temperature, humidity, food and light on the metamorphic changes in mutants of two species of the flour beetle, *Tribolium castaneum* (Herbst) and *T. confusum* (DuVal) were investigated in this study. Of these factors, temperature and humidity were of prime importance since they greatly influenced the development of each metamorphic stage of the insects. The temperature preference of each stage was noted and recorded.

The three mutants of *T. castaneum*, "Jet," "Sooty," and "Pearl," followed a uniform pattern in temperature gradient analyses. The successive metamorphic stages from eggs to pupae were found to develop progressively from cooler to higher temperatures. The adults of all mutants of *T. castaneum* preferred a temperature lower than that at which pupae were to be found.

In *T. confusum*, the "Black," "Ebony" and "New York" beetles followed a different pattern from that of the mutants of *T. castaneum*. Both the eggs and larvae of these two beetles were found in a closely related mean temperature. The pupae and adults were found at a higher temperature than were the eggs and larvae. However, there was little difference between the preferred temperatures for pupae and adults. The mutant "Ebony" of this species followed a pattern similar to that for *T. castaneum* mutants in that there was an increase in temperature preference from eggs to pupae. As for *T. castaneum* mutants, the adults of *T. confusum* also preferred a lower temperature than that of the pupae.

Egg and pupal development among the mutants of both species were distinct in regard to temperature preferences. Egg deposition proceeded at a higher temperature in beetles of *T. confusum*.

The adult mutants of *T. castaneum* have a lower mean temperature preference than the beetles of *T. confusum*. The larvae of the mutants of *T. castaneum* developed at slightly higher temperatures than those of *T. confusum*.

It was determined from this investigation that a relative humidity lower than 70 per cent, in a temperature gradient ranging from 14° to 40° C, was

[1] Accepted for publication May 2, 1969.
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a factor that accelerated the growth of *T. confusum* and retarded the growth of *T. castaneum* mutants.

No attempt was made to study the effects of food and light in any great detail.

Introduction and Historical Background

The wild types of the confused flour beetle, *Tribolium confusum* (duV.) and the red-rust flour beetle, *Tribolium castaneum* (Herbst) are destructive because they infest flour and other prepared cereal products. These insects are cosmopolitan in distribution and reported from almost every civilized country in the world. *T. confusum* is the more common pest in temperate regions while *T. castaneum* is a subtropical insect (11). The life cycles of the two parent types are well known. The time periods required for the development of various stages in the life cycle are as follows:

	Egg Stage (days)	Larval Stage (days)	Pupal Stage (days)	Total No. Days (to adulthood)
T. confusum	5.5	22.4	7.0	34.9
T. castaneum	3.8	22.8	6.2	32.8

From the data obtained from Good and Park several norms have been established, viz., 1) The life cycle of *T. castaneum* is shorter than that of *T. confusum;* 2) The life cycle for both species is shorter at higher temperatures; 3) The established period of time necessary for both *T. confusum* and *T. castaneum* to pass through their metamorphic cycle is roughly about one month at 29° C, but *T. castaneum* reaches maturity first. Environment factors affecting growth and development are temperature, humidity, and available food supply (11) (26).

Thus, the economic importance, worldwide distribution of the genus *Tribolium*, and the ease with which its natural habitat can be constructed under controlled environmental conditions make *Tribolium* spp. appear an important, practical and workable tool for studies of the geneticist and ecologist.

The genetics of *Tribolium* has been investigated by Sokoloff (32) Since the work reported here is primarily concerned with ecological factors, the review of literature will present only the background on these factors, such as light, temperature, humidity, water, food, and the general habitat.

Light: Light is a factor in the metamorphic cycle of *Tribolium* spp., but because of insufficient research, its importance cannot be established with any degree of certainty. Under laboratory conditions it was obvious that the adults are photo-negative; i.e., they are found loosely clustered in shaded portions of a container when subjected to direct light (16). Uncomfortable or undesirable temperatures and humidity will cause *Tribolium* spp. to move from the food medium into light but for the most part the beetles can be maintained indefinitely in complete darkness according to Chapman (5). Erdman studied the effect of X-Ray beams on these beetles to determine the sensitivity of *Tribolium castaneum* and *Tribolium confusum* at different stages in their life cycles. Also the destructive effects of X-Ray treatments have on *Tribolium* spp. has been investigated. A variety of wave lengths of the spectrum are being studied, but to date the flour beetle has not been subjected to their rays in a manner that could measure wave length effect on the organism.

Temperature and humidity: Early work on wood-ants by Herter (15, 16, 17) was conducted in an apparatus called a "Temperatureogel." Unfortunately the temperature gradient within the cage of the Temperatureogel varied with the room temperature. Some investigations of Bodenheimer and Schenkin (1) were undertaken with an apparatus similar to the one used by Herter. They repeated Herter's earlier experiment with the "flour weevil" *T. confusum.* They found that the beetle preferred a temperature of 24.7 to 26.5° C. if previously kept at a temperature of 15 to 18° C. However, beetles kept for a month in a constant temperature of 25° C preferred a zone between 9.11 and 10.74° C.

Following this early work several studies were made in the ensuing years of the relationships of temperature and humidity, the effect of constant and alternating temperatures, and the influence of temperature gradients on development of the various stages of *Tribolium.*

In 1930 Brindley (2) studied growth and development in *T. confusum* under controlled conditions of temperature and relative humidity. He used a temperature of approximately 30° C with a relative humidity of 73%. Under these ecological conditions he studied the weights and sizes (length and width) of 3 stages, namely, adults, larvae, and pupae. He also counted the number of eggs laid, as well as their length and width. From his observations the life cycle was found to be completed within 29 days at 30° C and 73% relative humidity.

In contrast to Brindley's findings, a Polish worker, Mikulski, (24) found errors in studies carried out at constant temperatures. In 1936 Mikulski studied the effect of constant and alternating temperatures on the survival of some developmental stages of *T. confusum.* He quoted Shelford (31) as having noted that different temperatures can have a different influence on an organism. Mikulski asserts it is an error to use a mean daily temperature as an index of the effect of temperature on development. Mikulski concluded that constant temperatures retarded development. Therefore, his experiments were undertaken to show the influence of alternating temperatures on survival and development, particularly with reference to (a) the mean temperature at different points on the thermometric scale, (b) the amplitude of the change of the temperature; (c) temperature limits. At extreme temperatures, i.e., in very low or very high temperature ranges, the behavior of the organism deviated from those expected for medial temperatures. For example, with a rise in temperature the rate of development increases up to a point, after which it decreases again. Mikulski concluded that *T. confusum* is a stenothermic animal, having a narrow, favorable thermal range which differs for each developmental stage and that this thermal range is better expressed by differences in survival. The optima are also slightly different, when comparing the constant and alternating temperatures. It is different for eggs and pupae. There is quite a narrow range in which development rises above 50%. For eggs this range lies above 25° to 30° C. At 32.5° C, 80% of the pupae survive. At a temperature of 22.5° C pupae developed well. 29.82% of the pupae survived at 22.5° C while only 25% of the eggs survived. Mikulski (24) found eggs of *T. confusum* and *T. castaneum* to be less resistant to constant temperatures, but *T. confusum* has a different resistance to symmetrical alternating and constant temperatures. Mikulski seems to think that *T. confusum* is insensitive to humidity and cites Holdaway (18) as his authority. In any case, the relative humidity fluctuated between 30 and 40% in his expeirment.

Howe's (20) work appears to verify Mikulski's conclusion that *T. confusum* is insensitive to humidity. Howe demonstrated that humidity has no effect on egg development of *T. confusum* and *T. castaneum*. He showed that eggs of *T. castaneum* did not hatch at 17.5° C or lower at any humidity, and that they did not hatch at 40° C at 10% relative humidity. *T. confusum* eggs did not hatch at 15° or 40° C at any humidity, but 60% of the eggs hatched at 37.5° C while the shortest period for egg duration in this species is at 35° C. The shortest period for egg duration in *T. castaneum* occurs at 37.5° C. In *T. confusum*, larvae failed to develop to pupae at 17.5° C with 10% R.H. and failed to develop in 20° C at 10% R.H., and also failed to develop in 37.5° C at 10% and 90% R.H. *T. castaneum* larvae failed to develop into normal adults at 20° and 40° C with R.H. at 30% and 90%. The larvae of *T. castaneum* produced pupae at 20° C and 70% R.H. but they do not become normal adults. In both *T. confusum* and *T. castaneum* larval development is affected by both temperature and humidity. *T. castaneum* larvae develop most quickly at 35° C in any humidity, but preferably at the higher humidities. The fastest larval development of *T. confusum* is at 32.5° C at highest humidities. The larval mortality is greatest at 37.5° C and 10% R.H. and at 20° C and 90% R.H. *T. castaneum* larval mortality is highest in situations of low humidity and low temperature. The pupal stage of this species while not affected by humidity is shortest at 37.5° C. *T. confusum* shortest period of pupal duration is the same as that of *T. castaneum* at 37.5° C. The optimum conditions for rapid development of *T. castaneum* is between 35 and 37.5° C and greater than 70% R.H. For *T. confusum* the fastest development lies close to 32.5° C at 70% R.H. and above. The life cycle is completed in about 25 days, while for *T. castaneum* the cycle takes 20 days. *T. castaneum* can complete its life cycle in a month at temperatures as low as 30° C, and at humidities of 30% R.H. Larval mortality was greatest at low temperature (22.5° C) and low humidity (10 and 30%) as well as at 40° C, and, in general, was highest at the lower humidities. At temperatures above 35° C mortality of *T. confusum* was more than 20% at all humidities and greater than 60% when R.H. was less than 50%. Mortality rate between 15–25% were recorded for *T. confusum* at low temperature and low humidity. The lower limits for complete development was between 17.5 and 20° C except at lower humidities where it rose above 20° C.

Holdaway (18) also studied the development of *T. confusum* at various relative humidities under constant temperature conditions. He found that the egg and pupal stages were almost the same duration throughout the entire scale of relative humidity. The length of the larval life, however, was shortened when humidity was increased. From the standpoint of per-cent mortality, however, he found that there was a greater survival of the larvae at high humidity but a reduced survival of eggs and pupae. The larvae, therefore, have their per cent survival increased and the length of time for development decreased by an increase of humidity, while the pupae and eggs do not have the time change but have the percentage greatly reduced by high humidity. However, the greatest difficulty in experiments using high humidities was controlling the growth of fungi. This could possibly have been avoided with use of silica gel.

The next reported work on temperature and humidity requirements of the flour beetle is concerned with establishment of a temperature "range" in which the insect prefers to live and can readily survive. This can only be established for the adult form *alone*. Deal (8) used a temperature gradient since he intended to prove that insects, given a preference will choose a "range" of temperatures rather than a "point." He stated that insects previously kept in a definite temperature for a given length of time with food available or not will choose a variety of "preferred temperatures." For example, an insect kept for a period of time, let us say a month, in one area at room temperature, when subjected to the temperature gradient area, will choose one temperature range. However, when kept in an environment at 27° C for a period of time previous to being subjected to the temperature gradient, the insect will choose an entirely different temperature. In each case the insects were without food which would be a factor influencing their physiological activities, since the normal metabolic functions of the insect may be hampered and its reactions thereby slowed. Food is no less an important factor than temperatures in altering the normal body activities of insects. *T. confusum*, when kept at room temperature, showed a definite preference for temperatures between 25–30° C but could be grown in temperatures as low as 10° C. In another experiment the beetles were kept for a month at a constant temperature of 27° C prior to going into the temperature gradient. The beetles were found in higher numbers at the cold end but some were found at ranges from 16° to 30° C. The greatest number of insects were found at a temperature 12° *lower* than room temperature. It is possible that the flour beetle, if kept for a period of time in a hot, dry environment, which is not exactly to its liking, would seek out an area which would first satisfy their greatest need, which would be moisture. Deal's results might be questioned as to the validity of his conclusions regarding the actual effect of his temperature gradient. Although his gradients were series of 5° C, he allowed stored beetles freedom of choosing the gradient, then proposed an explanation for their selection of the gradient. For instance, he says it is difficult to distinguish whether the flour beetles went to the cold end of the gradient because of preference or whether they wandered into the cold zone and were overcome, or trapped, before they could get away. He further states that insects go to a certain temperature in the gradient because they are attracted by the humidity there.

Graham (12) worked out temperature-preference determinations for adults of *T. castaneum* and *T. confusum*, using a temperature gradient ranging from 13.5° C to 30° C and a relative humidity gradient of 40 to 60%. He demonstrated that *T. castaneum* had a definite preference for warm temperature, the limit for migration seldom being beyond 29° C. The limit for distribution at the colder end of the trough was 14° C. Further, Graham stated that the intensity of reaction towards 28° C or 14° C is dependent upon the environmental temperature at which the population (*T. castaneum* and *T. confusum*) was previously kept and that a colder environment initiated a more intense warm-end reaction. He found that a *T. castaneum* population from a warm environment has a markedly more intense reaction toward 28° C than *T. confusum*. However, Graham made the assumption that temperature preference for both species was a transitory matter and at best preference for a temperature in which to live or to which to migrate was characteristic only for the particular population under investigation.

Temperature preferences varied according to sex, Graham (12) found that males had a slightly higher preference for the warm end of the trough, whereas females had a greater preference for 14° C. However, Graham stated that eggs are laid in appreciable numbers only when the female was at temperatures greater than 25° C so it was assumed that ovarian activity did not affect movement throughout the trough to other temperatures. But, since eggs are laid in greatest numbers when the female is at 25° C temperature, the species (*T. castaneum*) that has a greater preference for the warm end has the greatest potential for reproduction. When the total environmental average is 20° C, *T. castaneum* would have a greater advantage for survival over *T. confusum*. Also, according to Dick (9) movement of beetles (*T. confusum*) from various temperatures on a gradient, that is, from a colder to a warmer temperature, stimulated the production of ova.

Water: Roth and Willis (30) (35) have pursued extensive studies on water balance in *T. confusum* and *T. castaneum*. They found that *T. castaneum* lost water more rapidly than *T. confusum*. The females of *T. castaneum* reverse their dry reaction more quickly than the males; in other words, females lose more water than males. In *T. confusum* the dry reaction of males is reversed more quickly and the resultant wet reaction is more intense than that of females. Although both sexes of *T. confusum* lose water at about the same rate, the females tend to maintain a higher proportion of water to solids than the males from the 3rd to 7th days of desiccation. Given a choice, *T. castaneum* in normal physiological conditions will choose a lower humidity. It is further interesting to note that *T. castaneum* can lay eggs well at very low humidities.

Habitat, Food Sources: One of the major physical and chemical factors that operates to provide the complete environmental habitat for Tribolium spp. is food. The primary source of food is based on coexistence reactions, such as "cannibalism." But these are of relatively slight nutritional importance in terms of the complete population.

The beetle, Tribolium, can accommodate and adapt itself very readily to the available food. Chittenden (7) found Tribolium in snuff, baking powder, ginger, peas and beans. He found the insect in whole-wheat flour, bleached and unbleached white flour, rye, rice and barley flours, in corn meal and in oatmeal. Good (11) reported *Tribolium castaneum* and *Tribolium confusum* living in chocolate, spices (red pepper), various kinds of nuts and even feeding on specimens in an insect collection. However, Chapman (6) found that *Tribolium confusum* were not equipped to feed on whole grains since the mouthparts were not adapted to attack large, hard pieces of food. In studying the feed preferences of Tribolium, he observed that either coarse or fine flours were equally populated by the beetles. He further observed that wheat-germ satisfied the requirements for growth and transformation. Lerner, Sokoloff and Ho (22) did a food preference study for *Tribolium confusum* and *Tribolium castaneum* using a mixture of corn flour, rice flour, soy bean flour and whole wheat flour to which brewer's yeast was added. They subjected *Tribolium castaneum* and *Tribolium confusum* to each type of flour with and without yeast, as well as a mixture. The result was that both species preferred the mixed type containing the yeast. Their next preference was rice flour and whole wheat flour. The third preference was for corn flour, and the least chosen food was soybean flour.

Sweatman and Palmer (34) were the first to make a critical study of the vitamin requirements of *T. confusum*. They found that wheat embryo added to a synthetic medium consisting of casein, salts, fats and dextrine shortened the time of development of the organism considerably (from 65 days to 28 days).

The effect of temperature increase on the moisture content of the sub-strata in a closed container has been studied by a number of workers (1) (10) (21) (29). Several workers have determined equilibrium curves for grain moisture content (air Relative Humidity). In an open system, where nearly limitless quantities of air of a uniform R.H. are available, or where excess moisture is added or removed by chemical solutions, the per cent moisture content of the grain is determined only by air R.H. The moisture content (M.C.) is nearly unaffected by those temperature changes which are not accomplished by R.H. changes (12).

Rough calculations indicate that a closed system, containing flour of a moderate moisture content exposed to a temperature of 20° C in the region of 10° to 30° will react thus:

a) When the volume of air enclosed is 200 to 400 times the volume of the grain, the grain moisture content—air R.H. equilibrium is practically unaltered.

b) When the volume of air enclosed is approximately 500 times greater than the grain volume, the moisture content of the grain and the air R.H. are held at a comparatively lower state because of the increase in the air's initially large potential for water retention.

c) When the volume of air is less than approximately 100 times the volume of the grain, the point of equilibrium is increased because the moisture released by the grain is far in excess of that which the air can retain.

Most measures of grain greater than a few grams are of necessity enclosed with volumes of air much less than 100 times the grain volume. A possible example of the closeting of grain in storage, or in the laboratory, which results in the level of the R.M.-M.C. equilibrium being *raised* when the temperature is increased (12).

With the foregoing facts in mind regarding the effects of environmental factors on parent types of *Tribolium spp.*, the present study was undertaken to determine the preferred temperature for egg, larval, pupal, and adult stages of *Tribolium* mutants. This paper reports the establishment of the temperature range of each life form of three mutants of *T. castaneum* and for two mutants and one wild type of *T. confusum* and the deviations among these mutants from characteristics of their parent type.

Part II: Ecological Variations of Mutants of Tribolium castaneum, T. confusum and a Wild Type

Materials and Methods

For these experiments, four controlled environmental factors, as recommended by Park (27) were to be investigated: 1) Light; 2) Temperature; 3) Relative Humidity; 4) Volume, kind, and surface exposure of growth medium. Oxygen supply and CO_2 assimilation were not considered as necessary controlled factors in this study.

The growth chamber apparatus consisted of a pine-veneered rectangular box, 4′ × 4′ × 2′ in height. A fluorescent light attached to the ceiling was controlled by a timing device that permitted a 12-hour light period and 12-hour dark period. A piece of plexiglass 2′ × 4′, painted black, which was readily removable afforded easy access to the incubator. The floor of the box was covered with an asbestos sheet upon which there was a lead plate 3½′ long × 2½′ wide. The leaded plate was 1″ thick and had copper tubing embedded in it at one end. Six tubes, one inch apart, were supplied with flowing ice water from a refrigerator. At the other end of the plate two heat strips were soldered to the lead plate and electrically controlled by a simple thermostat. To prevent excessive heat loss the heating strips were covered with dry, flaked asbestos. With ice water flowing through one end of the lead slab and heat being supplied at the other end, a temperature gradient was maintained which ranged from 14° C at the cool end to 40° C at the heated end. The thermostat used to control the heat could be readily moved over the surface of the lead slab. Several preliminary experiments were made to determine the proper placement of the thermostat to ensure maintenance of maximum temperature levels at the hot end of the trough.

Relative humidity was maintained with glycerol and water. A small heat strip was soldered to the base of a metal container holding a glycerol-water mixture. An electric relay was used to maintain proper heat to control the evaporation of water. The water was stirred slowly but constantly. A small fan was placed in position to circulate the moist air

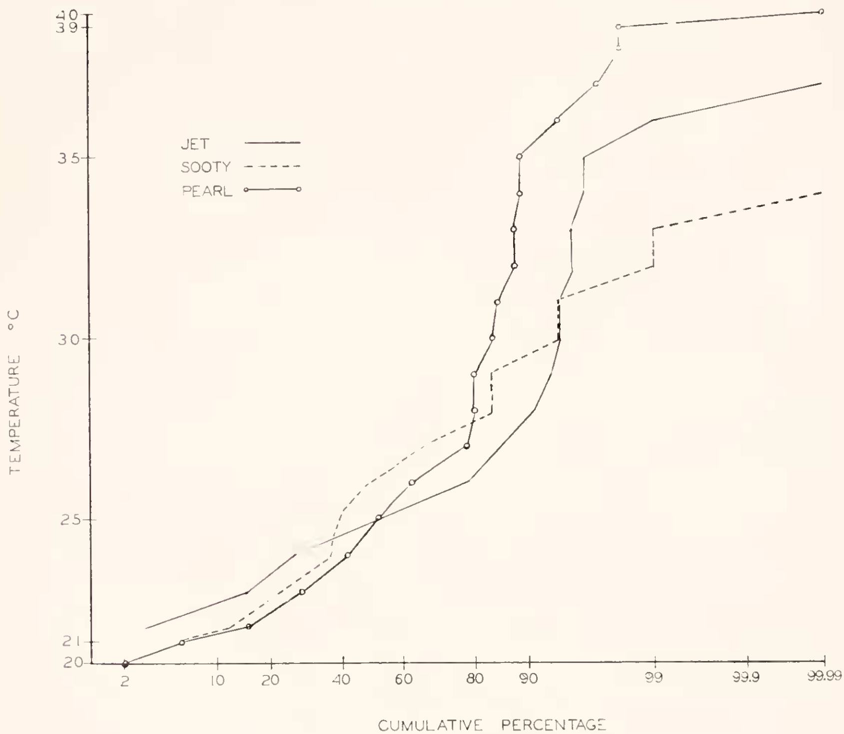

FIG. 1. Percentage deposition of eggs of three mutants of *Tribolium castaneum* at various temperatures.

but which would not disturb the flour and the insects. Using a hygrometer and a wet-bulb thermometer, readings were taken three times each day to determine R.H. Adjustments were made in the glycerol-water solution to elevate or lessen humidity percentage as needed.

When the proper R.H. was obtained and stabilized, copper food troughs less than 1 cm thick, 2″ wide and 2′ long were introduced into the incubator. Each copper trough was filled with food for the *Tribolium* spp.

This food consisted of 9 parts of unbleached flour and one part of powdered yeast. Periodic readings were made with an electronic thermometer to determine temperature gradient of food medium. For three days prior to the experiment, temperature and relative humidity were checked for reliability. The atmospheric temperature in the incubator was 27° C. The R.H. ranged from 55 to 75% within temperature ranges of 14° to 40° C with average R.H. being 60% immediately above the troughs.

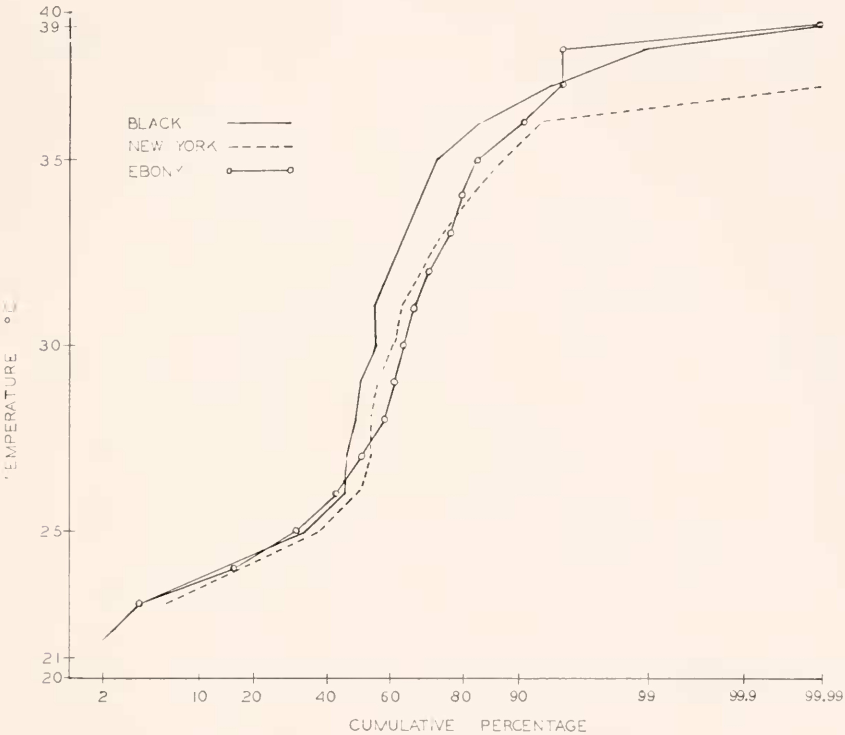

FIG. 2. Percentage deposition of eggs of three mutants of *Tribolium confusum* at temperatures ranging from 20° to 40° C.

When proper environmental conditions were established, populations of three mutants of *Tribolium castaneum* and 2 mutants and a wild type of *Tribolium confusum* were introduced in the incubator. Each genetic mutant population and one wild strain occupied a separate trough. Fifty males and fifty females comprised the initial population in each trough. After the mutants were placed in the feeding trough, the incubator was completely sealed and left undisturbed for one month.

At the end of the month flour samples from each trough were checked by means of an electronic thermometer to determine the temperature. At each degree of temperature in the food medium a sample of flour with its contents of *Tribolium* was taken with a small measuring spoon. The sample of flour was placed in a small jar. There were as many jars as degrees of temperature, each jar containing the measured flour sample. Counts of eggs, larvae, pupae and adult populations were made as fol-

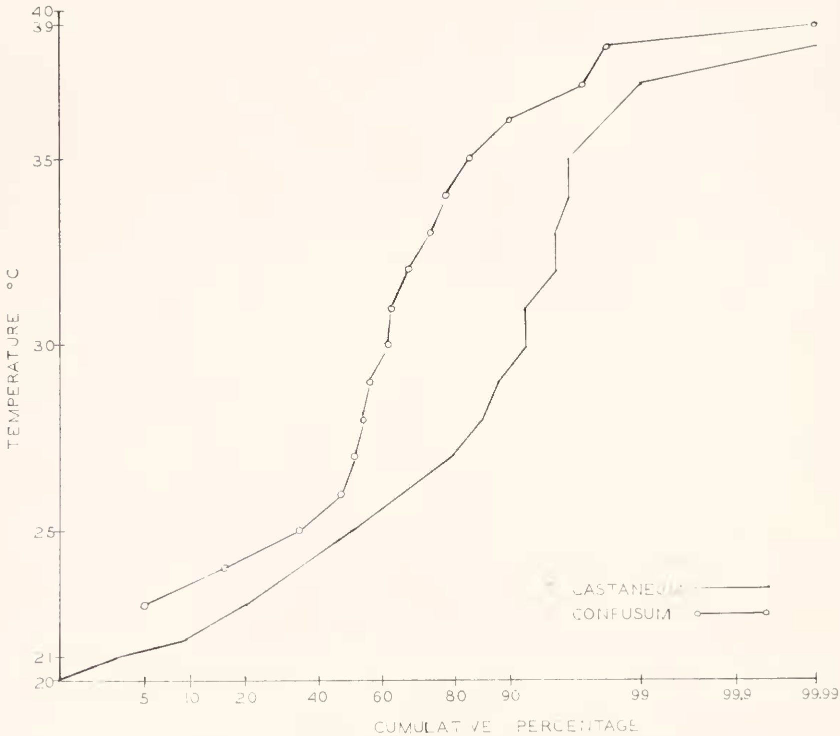

FIG. 3. Comparison of deposition of eggs of *Tribolium castaneum* and *Tribolium confusum* over a range of temperatures. Results indicate that egg deposition proceeded at a higher temperature in mutants of *T. confusum.*

lows: the contents of each jar was emptied into a sieve of No. 00 batting cloth (29 meshes/inch) which captured large larvae, pupae and adults of the insects. The small larvae and eggs were captured in a lower sieve. Using a dissecting microscope to examine the contents of the flour population in each stage in the cycle were counted.

This experiment was repeated three times. All conditions in each experiment were identical.

Results and Discussion

Temperature and humidity are two factors that influence the development of each metamorphic stage of *Tribolium* spp. A specific combination of these two ecological factors is necessary for the normal progressive development of each separate, metamorphic stage in the life cycle of the insect. However, temperature and humidity combinations that may be a

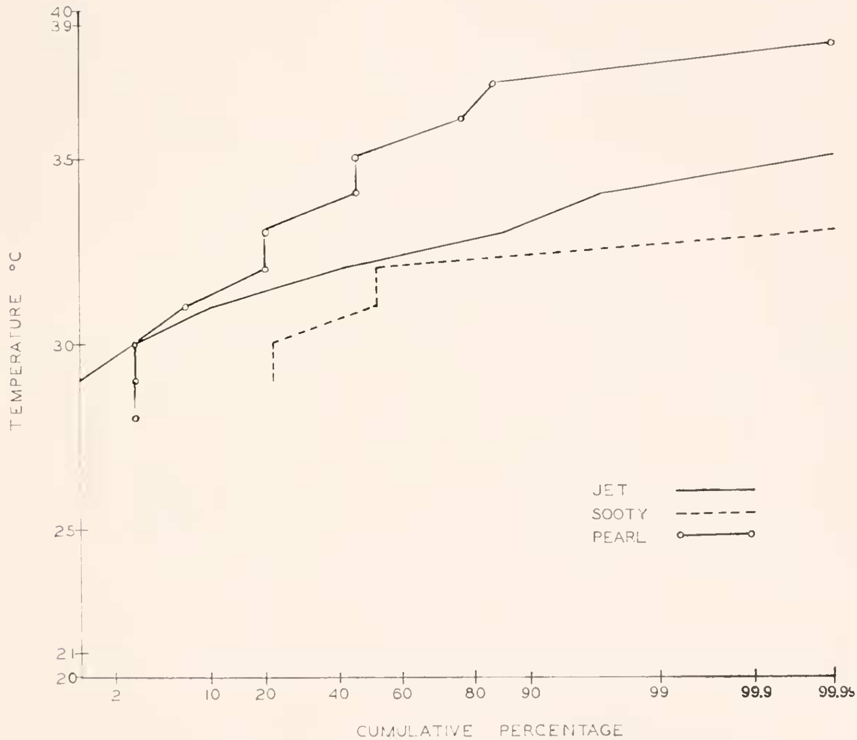

Fig. 4. Cumulative percentage deposition of pupae of mutants of *Tribolium castaneum* at various temperatures.

means of acceleration for one may retard the development of another life stage.

In this investigation humidity lower than 70%, with a temperature gradient ranging from 14° to 40° C was found to be the factor that accelerated the growth of *T. confusum* mutants and wild type strain but retarded the growth of the mutants of *T. castaneum*. All the data in this investigation were accumulated when the relaive humidity ranges from

40 to 60%. An approximate humidity of 60% was maintained throughout the investigation.

The temperature preference of the egg, the larvae, the pupa and the adult forms of six mutant beetles of *Tribolium* was the primary concern of this work. The mutants of *T. castaneum* were "Jet," "Sooty" and "Pearl." For *T. confusum* the two mutants & a wild type were selected and are identified as "Black," "New York" and "Ebony" (New York

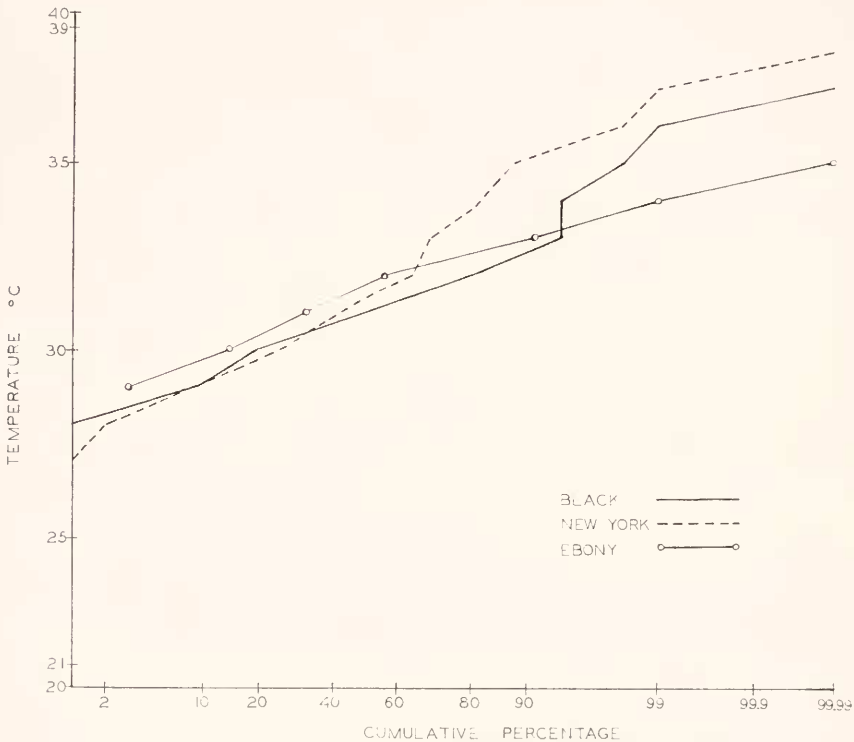

FIG. 5. Deposition of pupae of *Tribolium confusum* mutants over a range of temperatures from 20° to 40° C.

being the wild type). After a six-weeks interval from the time the adult imagoes were introduced into the troughs, data on life cycle forms found for each degree of temperature from 20° to 40° were recorded. These data are presented here.

The most significant fact in the temperature-gradient analyses was found in observations on the mutants of *T. castaneum*. There was found to be uniformity of mean temperature preferences by the successive stages of metamorphosis (the egg to the pupa) but a decrease in temperature was preferred by the adult form. This was found to be true of all three mutants of *T. castaneum*.

The "Black" mutant and "New York" wild type of *T. confusum* followed a pattern unlike that of the mutants of *T. castaneum*. Both the eggs and the larvae of these two mutants preferred a closely related mean temperature. The pupae and adults of "Black" and "New York" chose

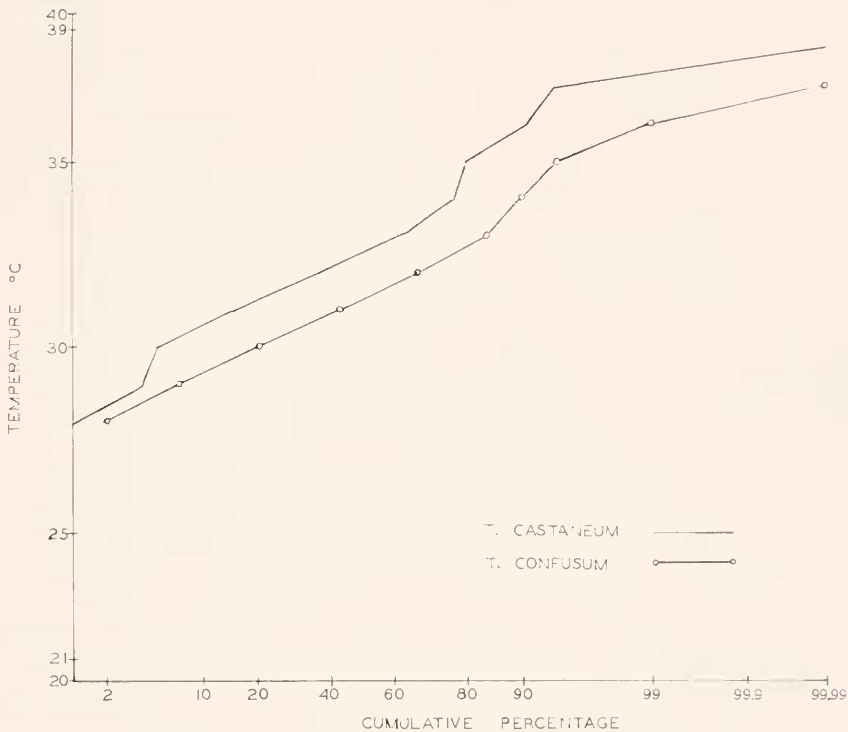

Fig. 6. Comparison of deposition of pupae of *Tribolium castaneum* and *Tribolium confusum* over a given range of temperatures shows that the pupae of *T. castencum* prefer the higher temperature.

a higher temperature than did the eggs and larvae but, as for the eggs and larval stages, there was but little difference between their preferred temperatures. The mutant "Ebony" of this wild type species *T. confusum* followed the pattern of the *T. castaneum* mutants with an increase in temperature from eggs to pupae. The adults chose a lower temperature than that of the pupae.

Temperature preferences for Egg and Pupae: The temperature preference of the eggs in relation to the temperature range of the pupae for all mutants of both *Tribolium* species is an important consideration. Egg and pupae states are immobile stages. It has been fairly well established that temperature is one of the ecological factors that determines their position *in situ*, especially for the pupae. What other factors are responsible for oviposition, other than temperature, have yet to be investigated.

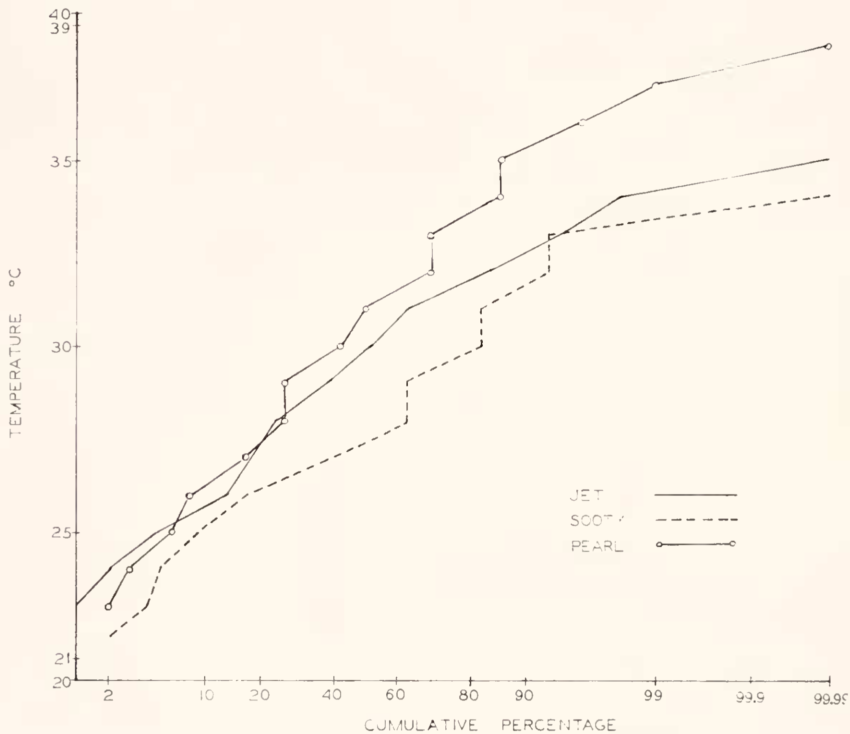

FIG. 7. Deposition of larvae of *Tribolium castaneum* mutants at various temperatures.

It would seem from the accumulated data-sheet that an analysis of their temperature differences was not necessary. There was very little overlapping of the minimum and maximum range in any of the mutants in these two stages of development. The mean temperature preference of the eggs of the mutant "Jet" was 25.82 + 2.81. The pupae of the same mutant was at 32.56 + 1.03. The mean temperature for the eggs of "Sooty," a *T. castaneum* mutant was 26.11 + 2.66; the pupae developed at 31.52

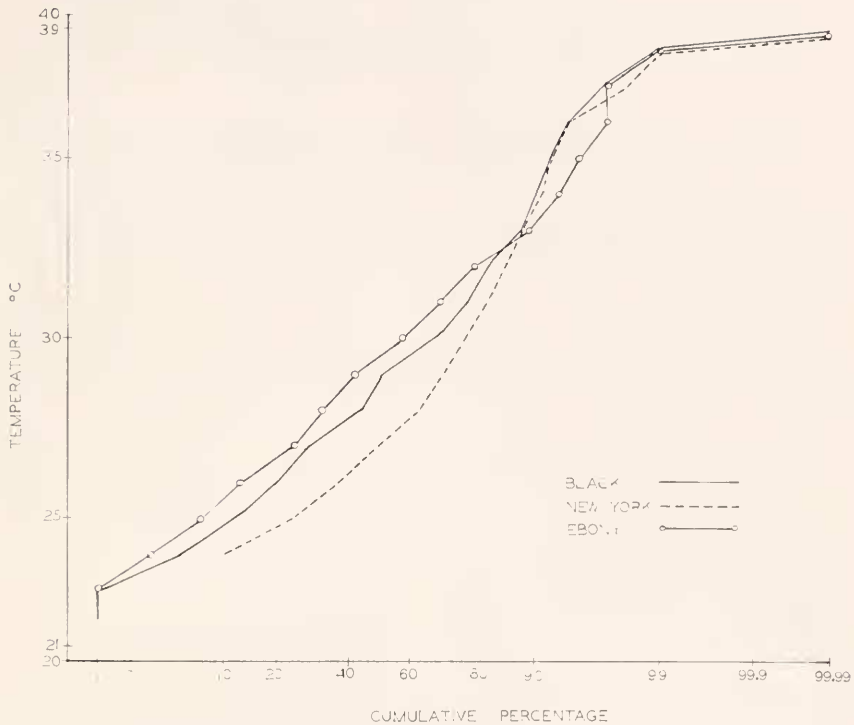

FIG. 8. Deposition of larvae of *T. confusum* mutants and wild type at various temperatures.

+ 1.62. The *T. castaneum* "Pearl" mutant had eggs at 26.39 + 4.60 with the pupae at 34.91 + 2.36.

The egg deposition for *T. confusum* beetles differed from the preferred temperature for the egg deposition for *T. castaneum* mutants in that eggs of *T. confusum* were all laid at a higher temperature. However, there was a distinct difference in temperature preference between eggs and pupae of *T. castaneum* (Table 1). The *T. castaneum* mutant "Pearl" had the widest temperature range variation of all the mutants studied.

TABLE 1. Temperature preferences for eggs and pupae of the T. castaneum mutants

Developmental Stage	Mutants		
	Jet	Sooty	Pearl
Eggs	25.82+2.81	25.11+2.99	26.39+4.60
Pupae	32.56+1.03	31.52+1.62	34.91+2.36

The greatest number of eggs of the *T. castaneum* mutant "Jet" were found at 25° C. "Sooty" laid the greatest number at 28° C and "Pearl" deposited the greatest number at 27° C. The beetles of *T. confusum* laid the greatest number of eggs at 25° C.

For the pupal stage of the mutants of *T. castaneum*, the greatest number of forms of "Jet" and "Sooty" were found at 33° C. The greatest number of pupal forms of the "Pearl" mutant were found between 35° and 36° C. The pupal range among the mutants of *T. castaneum* was from 28° to 38° C. The greatest concentration of pupae of these mutants were found to be between 31° to 34° C.

For the mutants & wild type of *T. confusum* the development of the pupal stage ranged from 26° to 39° with the greatest concentration of pupae between 30° and 34° C. This variation in number and temperature preference can be attributed to low relative humidity. This could account in part for the increased population of the mutants of this species as compared with the population number for the mutants of *T. castaneum* which thrives in an environment where the relative humidity is high. These data are in opposition to Mikulski's work (24) wherein he showed that *T. confusum* was insensitive to humidity changes.

The mutant "Black" (*T. confusum*) had the greatest number of pupae at 31° C; and "Ebony" had the greatest number of pupae at 33° C.

Although the prime purpose of this investigation was to establish the temperature preference of the four stages in the development of "mutant" insects of *Tribolium* species, one cannot overlook comparison of the results with work done with the wild-type insects of *Tribolium*. A search of the literature did not reveal that any temperature-humidity studies had ever been done using these mutant types. Of the mutants studied, "Black" (*T. confusum*) exhibited the highest mean-temperature preference, 29.93 + 5.26. This conforms closely to Mikulski's (24) findings in which he observed that the highest rate of survival of the eggs of *T. confusum* was between 25° and 30° C. He also noted that 80% of the pupae survived at 32.5° C, whereas egg survival at this temperature was zero.

However, contrary to Graham (12) who observed that eggs are laid at temperatures greater than 25° C, all the mutant beetles under consideration laid eggs in considerable numbers at temperatures between 23–24°. All the mutant beetles of *T. castaneum* deposited eggs in a lesser number in an area of 22° C. The mutants of the same species, "Sooty" and "Pearl," deposited eggs at a temperature of 21° C and the mutant "Pearl" deposited eggs at 20° temperature. The mutant "Black" of *T. confusum* also deposited eggs at 20° temperature.

(To be concluded in the September issue, p. 237)